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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application Of	: Group Art Unit 1644
LOUIS D. FALO, JR. and CHRISTINA M. CELLUZZI	: Examiner F. VanderVegt
Serial No. 09/208,549	: Attorney Docket No. 214001-00705
Filed December 9, 1998	:
Entitled	:
INDUCTION OF TUMOR AND VIRAL IMMUNITY USING ANTIGEN PRESENTING CELL CO-CULTURE PRODUCTS AND FUSION PRODUCTS	:

DECLARATION OF LOUIS D. FALO, JR.

I, Louis D. Falo, Jr., being duly sworn, depose and state:

1. I am a co-inventor of the captioned patent application. The application was filed with the United States Patent and Trademark Office on December 9, 1998. The application is a divisional of Serial No. 09/030,985, filed February 26, 1998, which claims priority to provisional Application No. 60/039,472, filed February 27, 1997.

2. Prior to October 3, 1996, the invention embodied in the current claims was completed in this country. To establish this date of completion, enclosed is a University of Pittsburgh Disclosure of Invention form, attached at Tab A that was completed and submitted to the University of Pittsburgh Technology Transfer Office. The document indicates that the invention was complete at least by the time of filing the disclosure. More specifically, the "Description of Invention" section provides details as to the invention as claimed and test results demonstrating the feasibility of the invention.

3. All of the dates redacted from Section A1 of the enclosed Disclosure of Invention are before October 3, 1996.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

11/17/00
Date

Louis D. Falo, Jr.

UNIVERSITY OF PITTSBURGH

DISCLOSURE OF INVENTION

When completed and signed, return original to the Office of Technology Transfer and Intellectual Property, 911 William Pitt Union (648-2206), and send one copy to your Dean. Attach additional sheets as needed.

DESCRIPTIVE TITLE OF INVENTION:

Induction of tumor immunity by using Dendritic cell-tumor cell fusion of coculture.

Inventor(s):

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A. INVENTION DEVELOPMENT AND DESCRIPTION:

1. Development of Invention

Item	Date	Place	Identify Corroborating Persons or Records
a. First Date of Conception		DFCI	Notes, LDF
b. First Disclosure to Others		U of Pitt	Lab notebooks
c. First Written Description		U of Pitt	Notebook, LDF
d. Completion of First Model		U of Pitt	Lab Notebook, CMC
e. First Successful Operational Test		U of Pitt	Lab Notebook, CMC

DISCLOSURE OF INVENTION

2. List any past or contemplated publication or oral presentation of the invention. Attach copies of any publications, abstracts, etc.

Date	Type of Publication
	Meeting abstract - Society for Invest. Derm

3. Attach a description of the invention, including:

a. possible applications; b. novel or unusual features; c. test data; d. advantages over currently available technology.

See Attached Sheets

4. List any related developments by others. Attach copies of relevant publications by others, if any

Guo et al: Science, 263:518-520, 1994.

B. SPONSORSHIP OR OTHER SUPPORT:

1. Government related grant(s)/contract(s) under which developed, if any:
(Attach copy of Notice of Award for each.)

	Sponsor	Grant/Contract No.	Grant/Contract Amount
1.	None		
2.			
3.			

2. Non-Government financial support, if any:

	Sponsor	Type of Agreement*	Amount of Support
1.	Univ. Derm. Associates		$30, 000
2.			
3.			

* (Consulting, Research, etc.)

3. Non-University resources (including biological materials received from others) and facilities used in development (including those of University-affiliated hospitals or other affiliated entities). Specify suppliers of any biological or other materials used to develop invention. Include time period and extent of use:

None

ISCLOSURE OF INVENTION

COMMERCIAL POTENTIAL:

1. List any companies which have expressed an interest or may be interested in licensing the invention for further development and sale:

Company (include address)	Contact Person and Telephone Number
a.	
b.	
c.	

2. Are you a shareholder, officer, director or consultant of any of the above companies? If so, explain.

lease date and sign this report and have two witnesses who understand its subject matter also sign as indicated:

Signature - Full Name of Inventor(s)	Date of Signature
[signature]	
Christina M. Allegri	

Date explained to and understood by me	[signature] Signature of Witness (Full Name)	Date of Signature
Date explained to and understood by me	[signature] Signature of Witness (Full Name)	Date of Signature

REVIEWED AND APPROVED:

[signature] Department Chair or Senior Unit Administrator	Date of Signature

ASSIGNMENT

In consideration of my/our rights and obligations under the University of Pittsburgh Patent Policy and Procedures and of One Dollar ($1.00) and other good and valuable consideration, the receipt of which is hereby acknowledged, and intending to be legally bound hereby, the undersigned inventor(s) hereby sell, assign and transfer to the University of Pittsburgh - of the Commonwealth System of Higher Education, a non-profit corporation of the Commonwealth of Pennsylvania (Assignee), its successors, assigns and legal representatives: 1) my/our entire right, title and interest, for all countries, in and to any and all inventions, developments and improvements (collectively "Inventions") which are disclosed in the appended Disclosure of Invention entitled Induction of tumor immunity by using Dendritic cell-tumor cell fusion of coculture.
and signed on _____; 2) in and to any and all United States and foreign patent applications including all divisional, continuing, substitute, renewal, reissue and all other applications for Letters Patent which have been or shall be filed on any of said Inventions, disclosed in the Disclosure of Invention; and to all original patents, reissued patents, confirmation patents and patents of addition which have been or shall be issued on said Inventions; and 3) in and to any and all continuations, extensions, and/or renewals thereof, including all priority rights under the International Convention associated therewith for each country of the Union.

I/We agree that Assignee shall have and to hold the interests herein assigned to the full ends of the terms of said Letters Patent and any and all divisions, reissues, continuations, renewals, and/or extensions thereof, respectively, as fully and entirely as the same would have been held and enjoyed by me/us had this assignment not been made.

I/We hereby authorize and request the United States Commissioner of Patents and Trademarks and any corresponding foreign officer to issue to Assignee, its successors, assigns and legal representatives, any and all United States and foreign Letters Patent on said Inventions disclosed in the Disclosure of Invention.

I/We agree that, when requested, without charge to, but at the expense of Assignee, I/we will: 1) execute, for all countries, all divisional, continuing, substitute, renewal, reissue and all other patent applications on any and all said Inventions; 2) execute all rightful oaths and other papers; 3) communicate all facts known to me/us relating to said Inventions and the history thereof; 4) testify in all legal proceedings; and 5) generally do everything possible which Assignee, its successors, assigns and representatives shall consider desirable for aiding in securing, maintaining and enforcing proper patent protection for said Inventions, and for vesting title therein in Assignee, its successors, assigns and legal representatives.

I/We covenant with Assignee, its successors, assigns and legal representatives that I/we have made to others no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed, and that the undersigned have full right to convey the same.

Witness:

Typed/Printed Name of Inventor

Signature of Inventor

Date:

Witness:

Typed/Printed Name of Inventor

Signature of Inventor

Date:

Witness:

Typed/Printed Name of Inventor

Signature of Inventor

Date:

Witness:

Typed/Printed Name of Inventor

Signature of Inventor

Date:

CONFIDENTIAL:
Disclosure of Invention
"Induction of Tumor Immunity Using Dendritic Cell - Tumor Cell Fusion or Co-Culture"
Falo/Celluzzi

3. Description of Invention

Problem:

CTL(Cytotoxic T-lymphocytes) have been recognized as a critical component of antigen-specific immune responses to tumors. For CTL induction and expansion to occur, the CTL receptor must recognize a ligand consisting of a self MHC-class I molecule and a peptide antigen derived from proteins synthesized within the tumor target cell(1,2). In addition, the peptide-MHC class I complex must also be presented in the context of costimulatory factors typically provided through interactions with a professional APC (3). Immunizations designed to stimulate tumor-specific CTL responses generally require: 1). the identification of tumor specific antigens presented by MHC class I molecules, and 2.) a method for delivering the tumor antigen in a manner that will induce antigen-specific CTL immunity. Although antigenic tumor peptides are being defined for some tumors, and some "shared" tumor antigens have been described, it is currently not feasible to identify unique tumor antigens for each potential patient. The invention we describe obviates the need to identify tumor specific antigens and provides a mechanism which delivers tumor antigens into the MHC class I restricted antigen processing pathway of professional antigen presenting cells(APCs).

Invention:

Though tumor cells express antigens which can be targeted by CTLs, tumor cells do not themselves stimulate CTL immunity, presumably because they are incapable of providing antigen in the appropriate context of costimulation. Dendritic cells, on the other hand, express a variety of costimulatory molecules and cytokines, and are the most potent APCs for CTL induction(3). Through our technology, dendritic cells are fused to tumor cells. In this fashion, a complete array of tumor antigens are delivered to the endogenous pathway of dendritic cells for class I specific presentation and CTL stimulation. Similarly, from another perspective, tumor cells become more immunogenic by becoming more like professional APCs through fusion of the APC with the tumor cell(4). The product of the fusion should express properties of both the APC and tumor which are capable of priming a CTL response, resulting not only in its own tumor destruction, but also in the destruction of other tumor cells that express similar antigens.

Supporting Data:

To investigate the feasibility of this strategy, we have fused DCs with the murine melanoma, B16, or the 3LL Lewis lung carcinoma, to determine the ability of fixed cells to induce CTLs and protection against a tumor challenge in mice. Naive syngeneic animals were immunized with irradiated cells after tumor-DC fusion. Our results demonstrate the following points:

1.) This technology results in the induction of tumor specific lytic activity in immunized mice(Figs. 1 and 2). Splenic effector cells from syngeneic naive mice immunized with irradiated fused cells lyse tumor cells used for immunization, but not an irrelevant syngenic tumor cell line(Fig 1.). Immunization with either irradiated tumor cells alone, or irradiated dendritic cells alone does not induce lytic effector cells(Fig. 1). The immunogenicity of this technique is generalized to 2 unrelated tumor types (B16 melanoma, and 3LL Lewis lung carcinoma)(Fig 1.). Effector cells in immunized animals express the phenotype (CD8+, Thy1+) of cytotoxic T-lymphocytes(Table 1).

2.) Immunization with fused cells, but not tumor cells alone, or dendritic cells alone, protects animals from tumor challenge(Fig. 2.). This is generalized to two tumor types (B16 and 3LL). Furthermore, immunization with DC-tumor fusions results in regression of established tumors (Fig. 3).

Together, these experiments demonstrate that this technology can be used to induce potent tumor specific CTL-mediated immunity. In tumor models, immunization by these methods results in both protective tumor immunity, and effective immunotherapy of established tumors. The technology is unique in that it 1.)obviates the requirement for identification of individual tumor antigens, 2.)provides a mechanism for antigen delivery into the appropriate antigen-presentation pathway of professional antigen presenting cells, and 3.)delivers the entire array of antigens produced by a tumor cell to the most potent antigen presenting cells, providing a mechanism for broad polyvalent immunization. Existing technology closest to the technology disclosed here is the fusion of activated B-cells with tumor cells described by Guo et al (4). Advantages of our technology over described B-cell fusions include 1.) Dendritic cells are the most potent APCs identified thus far, 2.) DCs can be readily obtained from peripheral blood precursors by established protocols, 3.) DC-based fusions do not require manipulations necessary for activation and maintenance of B-cell APC activity. Like Guo et al(4), we have not yet definitively evaluated the possibility that unfused cells in the fused-cell population are also immunogenic. For this reason we include co-culture as a technology for enhancing immunogenicity in this disclosure.

References:

1. Yewdell, J.W. and Bennink, J.R. Cell biology of antigen processing and presentation to MHC class I molecule restricted T lymphocytes. Adv Immunol, *52*: 1-42, 1992.

2. Townsend, A. and Trowsdale, A. The transporters associated with antigen presentation. Sem. Cell Biol., *4*: 53-61, 1993.

3. Steinman, R.M., Witmer-Pack, M., and Inaba, K. Dendritic cells: antigen presentation, accessory function and clinical relevance. Adv. Exp. Med. Biol., *329*: 1-9, 1993.

4. Guo, Y., Wu, M., Chen, H., Wang, X., Liu, G., Li, G., Ma, J., and Sy, M. Effective tumor vaccine generated by fusion of hepatoma cells with activated B cells. Science, *263*: 518-520, 1994.

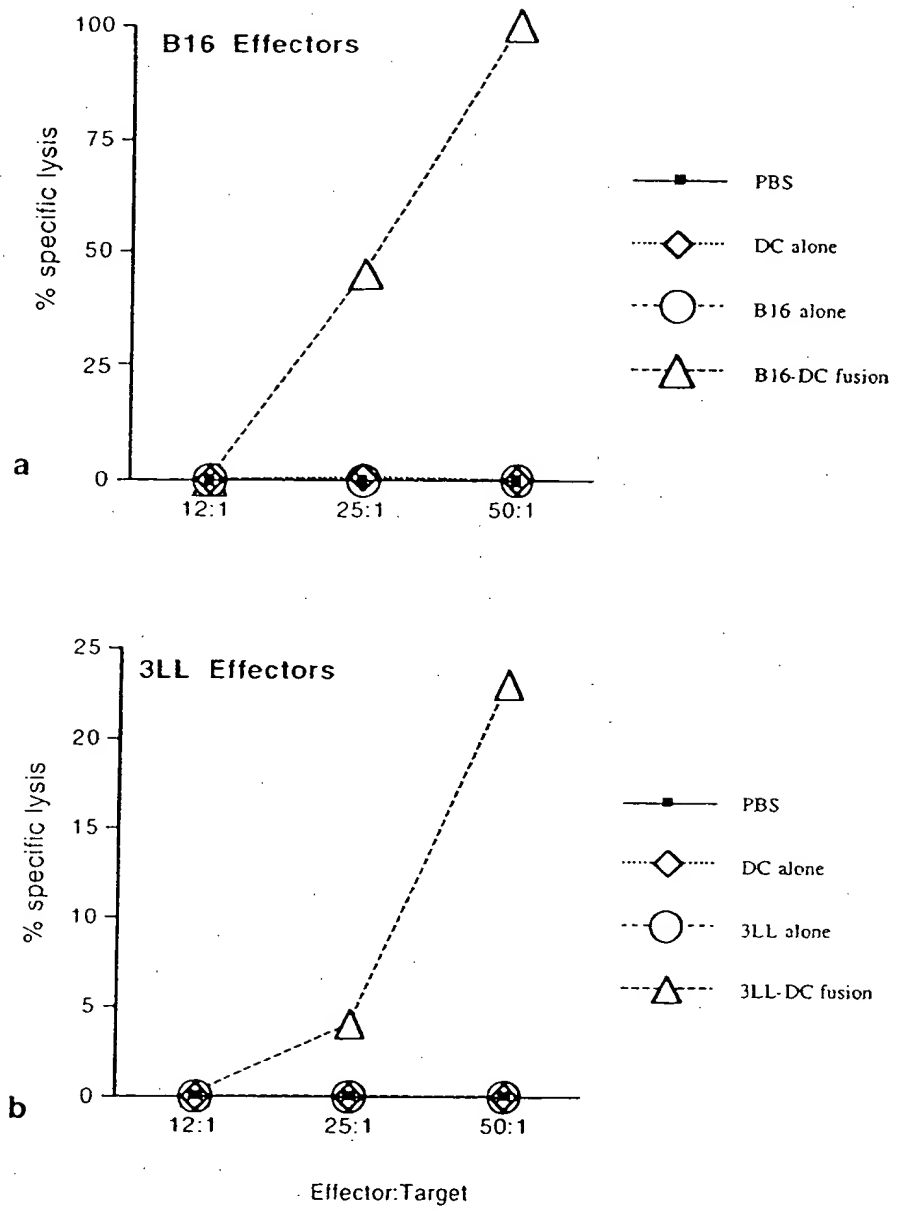

Figure 1: Immunization with PEG-fused bone marrow-derived dendritic cells (DCs) plus tumor cells induces a CTL response in mice. (a), Immunizations using B16 (C57BL/6 melanoma (H-2^{b}) (ATCC, Rockville, MD) or (b), 3LL (Lewis lung carcinoma) cells. C57BL/6 mice were immunized s.c. in the bilateral flanks with PBS (solid squares), 3 x 10^{5} irradiated tumor cells alone (B16 or 3LL, circles), 1.7 x 10^{6} irradiated DCs given alone (diamonds) or 1.7 x 10^{6} DCs PEG-fused with B16 or 3LL (6:1, triangles). 7-10d after immunization, splenocytes (30 x 10^{6}), were harvested and restimulated by coculture with irradiated B16 or 3LL cells (20,000 rad, 7.5 x 10^{6}) for 5 days. After this time, cytotoxicity assays were performed. Briefly, 18h prior to assay, target cells (immunizing cells B16/3LL or irrelevant targets; BL6-8 (H-$2K^{b}$, H-$2D^{b}$ B16-derived melanoma) or EL4 (C57BL/6 T lymphoma (H-2^{b}), were labelled by incubation in RPMI with ^{51}Cr (100 µCi; NEN, Boston, MA) at 37°C and washed extensively before use. Splenocytes (100µl) were cocultured at 37°C in 96-well round bottom plates for 4h at the indicated ratios in RPMI with the ^{51}Cr-labelled B16, 3LL or irrelevant targets cells (2 x 10^{4} targets/well). 100 µl of supernatants from triplicate cultures was collected and counted. Data points are expressed as the mean percent specific release of ^{51}Cr from target cells and are the mean of triplicate cultures. Less than 5% lysis was seen in irrelevant (BL6-8 or EL4) targets at all ratios (not shown).

Table 1

Immunization with PEG-fused dendritic cells and B16 (H-2b) melanoma induces CD8+ CTLs

	% specific lysis of B16 (H-2b) target cells from splenocytes:			
Effector:Target	CD4-	CD8-	NK-	Thy1-
12:1	0	0	0	0
25:1	68	4	51	0

Mice were immunized with PEG-fused DCs and B16 melanoma cells as described in Figure 1. On day 7 splenocytes were harvested and restimulated with irradiated B16 cells. mAb+C' -treated splenocyte effectors were depleted for CD4+, CD8+, Thy 1.2+ or NK+ cells and assayed on 51Cr labelled B16 targets as described in Figure 1. <5% lysis was observed on BL6-8 (H-2b-) or EL4 irrelevant targets (not shown). Lysis= +/- 2% SEM

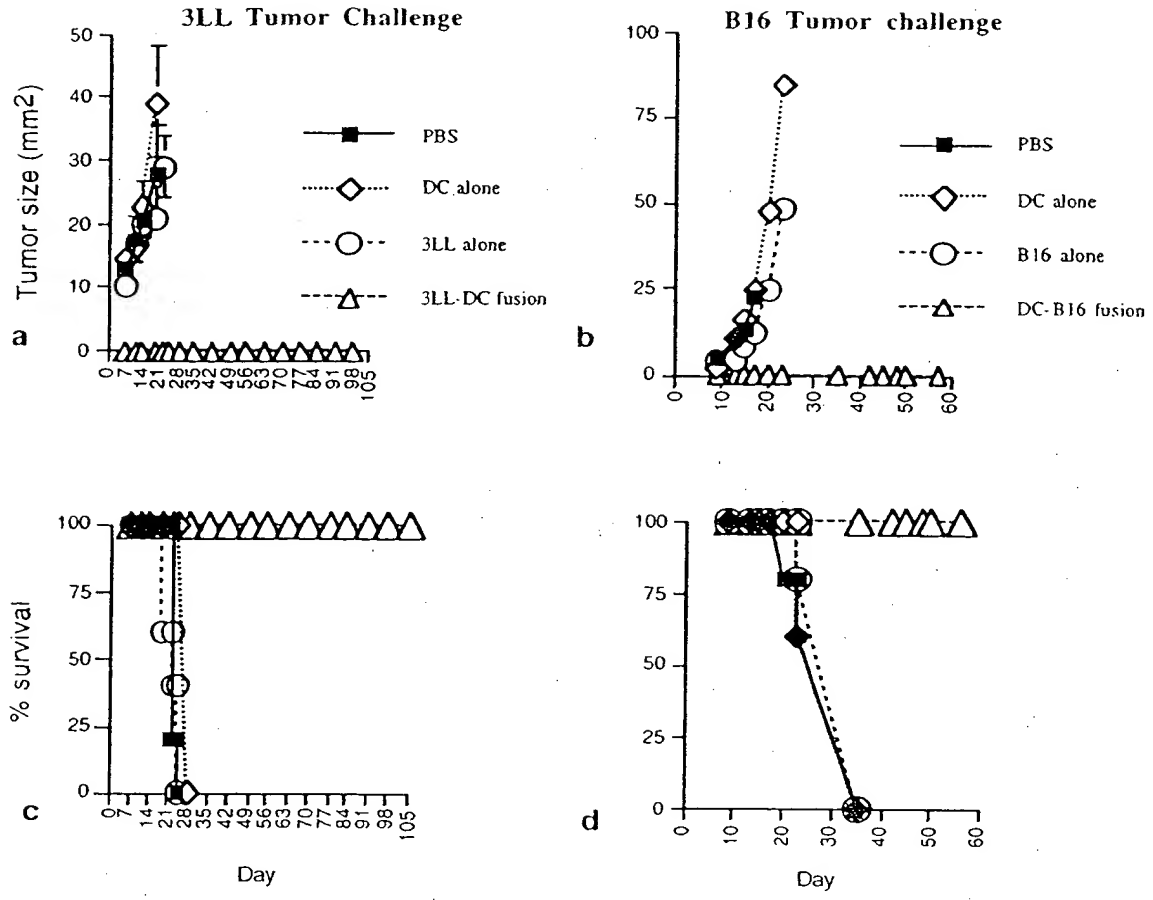

Figure 2: Mice immunized with DCs fused with tumor cells are protected from tumor challenge. C57BL/6 mice were immunized s.c. (as described in Figure 1) with PBS (solid squares), 3 x 10^5 irradiated tumor cells alone (circles), 1.7 x 10^6 irradiated DCs given alone (diamonds), or 1.7 x 10^6 DCs PEG-fused with B16 or 3LL tumor cells (6:1, triangles). On day 7, mice were challenged intradermally with 2.5 x 10^4 B16 or 3LL tumor cells/side bilaterally on the abdomen (**a,b**). Tumor size of each tumor was measured approximately three times weekly and is reported as the area +/- SEM in square millimeters. Tumor size is plotted to the day when the first animal in each group dies or is sacrificed. Survival (**c,d**) is recorded as the percentage of surviving animals. All experiments included 5 mice per group and were repeated. Mice becoming moribund were sacrificed.

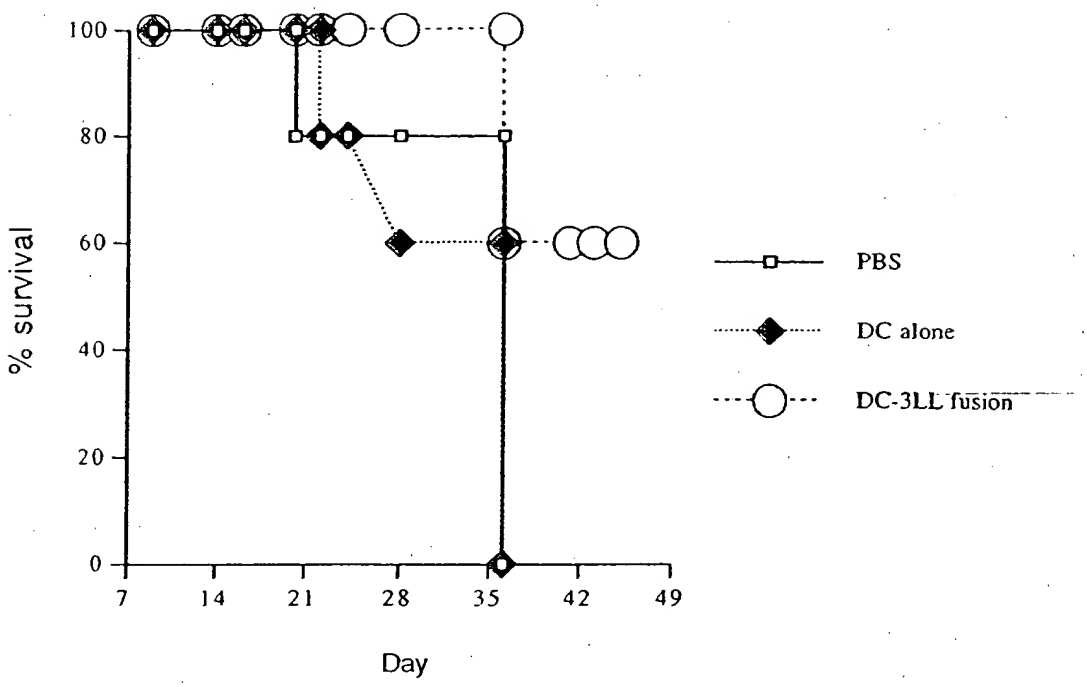

Figure 3: Treatment with dendritic cells (DC) fused with 3LL cells causes regression of pre-existing 3LL tumors in mice. Naive mice were challenged i.d. with 3LL (described in Figure 2). Mice presented tumors and were treated on day 9 with PBS, DC alone or DC PEG-fused with 3LL (described in Figure 2). Survival for each of the treatment groups was assessed as described in Figure 2. In surviving mice, there was no evidence of tumor development.